

## ABSTRACT

This invention comprises a novel approach to the assessment of antigen-specific T cells that quantitates and characterizes these cells with unprecedented clarity, and importantly, because it is performed in whole blood, is amenable to routine use in the clinical immunology laboratory. The methodology offers an improved flow cytometric intracellular cytokine assay in whole blood that can simultaneously measure multiple T cell subsets expressing multiple cytokines from a single whole blood culture. Evaluation of whole blood antigen specific cytokine responses has the important advantage of assessing T cell activation in the presence of ALL types of MHC autologous antigen presenting cells present in the native sample. It also has the advantage of enabling a culture system (whole blood) which can reflect effects of systemic environments (i.e. drug augmentation or suppression) on T cell responses to specific stimuli including antigen. by either culturing in the presence of such drug or analyzing the blood of a human or animal receiving such drug.